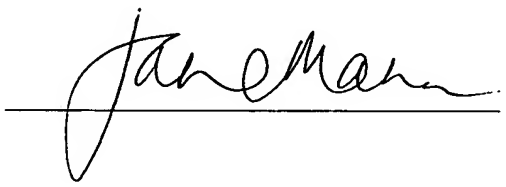
DECLARATION

I, Jane Roberta Mann, B.A., a Translator, of Frank B. Dehn & Co., 59 St Aldates, Oxford OX1 1ST, England, do declare that I have a competent knowledge of the English and German languages and that the document that is annexed hereto is a true and accurate translation of the German text of the U.S. Provisional Application Serial No. 60/503,317 filed September 16, 2003.

I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true.

I acknowledge that wilful false statements and the like are punishable by fine or imprisonment, or both [18 U.S.C. 1001] and may jeopardize the validity of the application or any patent issuing therefrom.



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Use of Angiotensin II Receptor Antagonists

The invention relates to the field of the angiotensin II receptor antagonists and relates to their use for treating people in whom diabetes has been diagnosed or who are suspected of prediabetes.

Type 2 diabetes mellitus is the manifestation of two pathophysiological phenomena, namely a reduced secretion of insulin from the beta cells of the pancreas and insulin resistance in the target organs of the liver, skeletal musculature and fatty tissue. As a rule there is a complex disruption of both components. The disease is diagnosed as fasting hyperglycaemia, i.e. the blood sugar concentration after 10-12 hours' fasting is above the threshold of 125 mg of glucose per dl of plasma. Controlled treatment of manifest type 2 diabetes can be achieved using compounds of the category of the thiazolidinediones (glitazones). These compounds improve the utilisation of circulating insulin and thus result in a lowering of the blood sugar levels (insulin sensitisers). At the same time the increased insulin levels are reduced by feedback mechanisms and in this way the load on the pancreas is relieved. Insulin sensitisers such as troglitazone, rosiglitazone or pioglitazone develop this activity by binding to specific nuclear receptors known as PPAR-gamma (Peroxisomal Proliferator Activated Receptor).

As every second type 2 diabetes patient show signs of coronary heart disease at the time of diagnosis, for example, the causes of diabetes are increasingly suspected to reside in a complex metabolic disorder which may be indicated by a number of risk factors such as abnormal glucose tolerance, increased fasting blood sugar, insulin resistance, high blood pressure, dyslipidaemia or centripetal obesity. The prevalence of insulin resistance is particularly marked in patients with hypertriglyceridaemia and low HDL-cholesterol. Reference is made to pre-type 2 diabetes, metabolic syndrome, syndrome X or insulin resistance syndrome. In a first phase a reduced insulin response by the target organs causes an increase in the pancreatic insulin secretion in order to keep the blood sugar level in the normal range. After a number of years of excessive or increasing insulin production there comes a time when the

insulin secretion by the beta cells of the pancreas cannot be increased any further. The phase of abnormal glucose tolerance then begins. The body can no longer absorb glucose peak values fast enough. Finally, if the fasting blood sugar remains persistently high, diabetes is manifest.

WO 95/06410 discloses the use of angiotensin II receptor antagonists for treating chronic inflammatory diseases including systemic autoimmune diseases. Diabetes is mentioned as one of a number of examples of systemic autoimmune diseases. The autoimmune diseases include type 1 diabetes mellitus which occurs mainly in young people under 30 years of age with a genetic predisposition, in whom insulitis occurs under the influence of various factors with subsequent destruction of the B cells so that the pancreas can only produce a little insulin or none at all. Type 2 diabetes mellitus is not regarded as an autoimmune disease.

The aim of the present invention is to provide a pharmaceutical composition which can be used both to treat manifest type 2 diabetes and to treat the first signs of the complex metabolic disorder of prediabetes and thereby prevent type 2 diabetes mellitus. Within the scope of the present invention it has now surprisingly been found that a few angiotensin II receptor antagonists and their salts not only act to reduce blood pressure, in known manner, but are also capable of increasing the expression of genes in a cellular system, the transcription of which is known to be regulated by the PPARgamma receptor. In order to ensure comparable conditions this effect is observed and quantified within the scope of the present invention by means of a stably transformed cell line (cf. Example 2). The cells used are CHO cells which are the result of transformation with two gene constructs. The first of these constructs codes for the luciferase gene from *Photinus pyralis* (de Wet JR, Mol Cell Biol (1987) 7:725) under the control of a synthetic promoter with a five-fold repeat of a yeast Gal4 binding site (cf. GeneBank Sequence AF058756). The second construct codes for a fusion protein consisting of the ligand binding domain of the human PPARgamma2 transcription factor (cf. GeneBank Sequence U79012) and the yeast GAL4 DNA binding domain (Amino acids 1-147; Sadowski I, Nucleic Acids Res (1989) 17:7539).

The induction of the transcription of PPARgamma-regulated genes is known from the thiazolidinediones used as antidiabetic drugs (e.g. rosiglitazone) and is brought about by their binding to the PPARgamma Receptor and its activation. Within the scope of the test system used here this effect may be quantified as an induced luciferase actvity of the transformed cell line. The same induction of a luciferase activity takes place with the angiotensin II receptor antagonists, contrary to expectation, not by the binding of the active substance to the PPARgamma Receptor. The induction is particularly marked for the active substance telmisartan. Binding of e.g. telmisartan to the PPARgamma receptor cannot be detected in various test systems. It is therefore presumed that the increase in the affinity of cofactor proteins for PPARgamma caused by an angiotensin II receptor antagonist such as telmisartan also leads to the recruiting of the cofactor proteins if there are no high-affinity synthetic PPARgamma ligands present. This then brings about activation of the transcription of genes regulated by the PPARgamma receptor, this activation being mediated by these cofactors. As the induction of these genes is responsible for the anti-diabetic activity of the thiazolidinediones it can be assumed that the induction of the same genes by angiotensin II receptor antagonists such as telmisartan results in a comparable anti-diabetic activity. Thus, these active substances are suitable not only for treating high blood pressure but also for treating and preventing type 2 diabetes mellitus.

The discovery of this new therapeutic effect of angiotensin II receptor antagonists and the salts thereof means that they can be used to produce a pharmaceutical composition for the treatment of people in whom type 2 diabetes mellitus has been diagnosed or who are suspected of prediabetes.

Type 2 diabetes mellitus manifests itself in a fasting blood sugar level exceeding 125 mg of glucose per dl of plasma; the measurement of blood glucose values is a standard procedure in routine medical analysis. If a glucose tolerance test is carried out, the blood sugar level of a diabetic will be in excess of 200 mg of glucose per dl of plasma 2 hours after 75 g of glucose have been taken on an empty stomach. In a glucose tolerance test 75 g of glucose are administered orally to the patient being

tested after 10-12 hours of fasting and the blood sugar level is recorded immediately before taking the glucose and 1 and 2 hours after taking it. In a healthy subject the blood sugar level before taking the glucose will be between 60 and 110 mg per dl of plasma, less than 200 mg per dl 1 hour after taking the glucose and less than 140 mg per dl after 2 hours. If after 2 hours the value is between 140 and 200 mg this is regarded as abnormal glucose tolerance.

If insulin resistance can be detected this is a particularly strong indication of the presence of prediabetes. Thus, it may be that in order to maintain glucose homoeostasis a person needs 2-3 times as much insulin as another person, without this having any direct pathological significance. The most certain method of determining insulin resistance is the euglycaemic-hyperinsulinaemic clamp test. The ratio of insulin to glucose is determined within the scope of a combined insulin-glucose infusion technique. There is found to be insulin resistance if the glucose absorption is below the 25th percentile of the background population investigated (WHO definition). Rather less laborious than the clamp test are so called minimal models in which, during an intravenous glucose tolerance test, the insulin and glucose concentrations in the blood are measured at fixed time intervals and from these the insulin resistance is calculated. Another method of measurement is the mathematical HOMA model. The insulin resistance is calculated by means of the fasting plasma glucose and the fasting insulin concentration. In this method it is not possible to distinguish between hepatic and peripheral insulin resistance. These processes are not really suitable for evaluating insulin resistance in daily practice. As a rule, other parameters are used in everyday clinical practice to assess insulin resistance. Preferably, the patient's triglyceride concentration is used, as increased triglyceride levels correlate significantly with the presence of insulin resistance.

Thus, there is a suspicion of prediabetes if the fasting blood sugar level is above the normal maximum level of 110 mg of glucose per dl of plasma but does not exceed the threshold of 125 mg of glucose per dl of plasma which indicates diabetes. Another indication of prediabetes is abnormal glucose tolerance, i.e. a blood sugar

level of 140-200mg of glucose per dl of plasma 2 hours after taking 75 g of glucose after a fast within the scope of a glucose tolerance test.

A triglyceride blood level of more than 150 mg/dl also indicates the presence of pre-diabetes. This suspicion is confirmed by a low blood level for HDL cholesterol. In women, levels below 40 mg per dl of plasma are regarded as too low while in men levels below 50 mg per dl of plasma are regarded as too low. Triglycerides and HDL cholesterol in the blood can also be determined by standard methods in medical analysis and are described for example in Thomas L (Editor): "Labor und Diagnose", TH-Books Verlagsgesellschaft mbH, Frankfurt/Main, 2000. A suspicion of prediabetes is further confirmed if the fasting blood sugar levels also exceed 110 mg of glucose per dl of plasma. If the blood levels measured are in the region of these threshold values, the ratio of the waist measurement to the hip measurement can be used as an additional aid to make the decision. If this ratio exceeds a value of 0.8 in women or 1 in men, treatment is indicated.

Angiotensin II receptor antagonists are particularly indicated for treating diabetes or suspected prediabetes if hypertension also has to be treated. This is the case if the systolic blood pressure exceeds a value of 140 mm Hg and diastolic blood pressure exceeds a value of 90 mm Hg. If a patient is suffering from manifest diabetes it is currently recommended that the systolic blood pressure be reduced to a level below 130 mm Hg and the diastolic blood pressure be lowered to below 80 mm Hg. To achieve these levels it may be indicated in certain cases to combine angiotensin II receptor antagonists with a diuretic or a calcium antagonist. The term "diuretic" included thiazides or thiazide analogues such as hydrochlorothiazides (HCTZ), clopamide, xipamide or chlorthalidone, aldosterone antagonists such as spironolactone or eperenone and also other diuretics suitable for treating high blood pressure such as furosemide and piretanide, and combinations thereof with amiloride and triamterene.

The present invention means that for subjects being treated for increased blood pressure, angiotensin II receptor antagonists such as telmisartan are indicated

whenever the development of prediabetes is to be prevented or manifest diabestes is to be treated.

In only 10% of all cases of elevated blood pressure (secondary hypertension) is it possible to determine an identifiable course such as e.g. kidney disease. As a rule, secondary hypertension can be remedied by treating and removing the cause. However, in almost 90% of all cases it is primary hypertension, the exact cause of which is not known and which therefore cannot be directly cured. The negative effects of elevated blood pressure can be reduced by changing lifestyle and correct treatment. The interaction of different risk factors or the combined occurrence of individual risk factors appear to cause high blood pressure. In particular, the combination of high blood pressure with disorders of the fat and sugar metabolism is observed to an increasing extent. These disorders are often unnoticed to begin with but can be recognised from increased blood levels of triglycerides and glucose and lower blood levels of HDL cholesterol. At a fairly advanced stage they can also be detected in slowly increasing corpulence. These disorders can be explained by increaing insulin resistance. The less effective the insulin, the more the fat and sugar metabolisms are disrupted. The combination of all these disorders in the last analysis increases the probability of contracting the sugar disease diabetes and dying prematurely of heart or vascular disease.

Estimates are based on the supposition that about a third of adults in those parts of the world with an excessive supply of food are affected by the combination of high blood pressure and disorders of the fat and sugar metabolism and that this number will continue to increase. Consequently there is a need for drugs which are capable of helping to slow down or stop the progress of the above-mentioned metabolic disorders at the earliest possible stage and at the same time to obviate the detrimental effects of increased blood pressure on the health.

The present invention also discloses a pharmaceutical composition which can be used both to treat hypertension and to treat manifest type 2 diabetes or the first signs of the complex metabolic disorder of prediabetes. Thus, the invention also includes

diabetes prevention in patients who are being treated for high blood pressure. If therefore a suitable angiotensin II receptor antagonist such as telmisartan is used immediately to control blood pressure as soon as one of the above-mentioned signs of prediabetes is present, the onset of manifest type 2 diabetes can be delayed or prevented.

Angiotensin II receptor antagonists which are suitable within the scope of the present invention are compounds for which binding to the PPARgamma ligand binding domain can be ruled out by *in vitro* tests (cf. Example 1), while they activate the expression of a stably transfected luciferase gene at cellular level, i.e. after the addition of a stably transformed PPARgamma reporter cell line to the culture medium (cf. Example 3).

Suitable angiotensin II receptor antagonists also exhibit

- no *in vitro* binding to the ligand binding domain of a human PPARgamma receptor, but lead to the
- induction of a luciferase activity when they are added to the culture medium of a stably transformed PPARgamma reporter cell line which
  a) expresses a fusion protein consisting of the ligand binding domain of the human PPARgamma transcription factor and the yeast GAL4 DNA binding domain and
  b) a luciferase gene under the control of a five-times repeated yeast Gal4 binding site.

The preparation of a PPARgamma reporter cell line of this kind is described in Example 2.

There is no *in vitro* binding to the ligand binding domain of the human PPARgamma2 receptor if it cannot be detected in an AlphaScreen (Ullmann EF et al, Proc Natl Acad Sci USA (1994) 91:5426-5430). Instead of an Alpha Screen, an SPA assay (Mukherjee R et al., J Steroid Biochem Mol Biol (2002) 81:217-225) or an NMR investigation (Johnson BA et al., J Mol Biol (2000) 298:187-194) may also be carried out. As a rule, binding to the receptor cannot be detected by any of these methods.

A comprehensive list of angiotensin II receptor antagonists can be found on pages 7-18 of WO 95/26188. Angiotensin II receptor antagonists are described *inter alia* in EP-A-253310, EP-A-323841, EP-A-324377, EP-A-420237, EP-A-43983, EP-A-459136, EP-A-475206, EP-A-502314, EP-A-504888, EP-A-514198, WO 91/14679, WO 93/20816, US 4,355,040 and US 4,880,804. Forms which are frequently mentioned are sartans, such as candesartan, eprosartan, irbesartan, losartan, olmesartan, tasosartan, telmisartan or valsartan. Those which are particularly preferred according to the present invention are irbesartan, losartan und telmisartan. The best results are clearly obtained with telmisartan and the salts thereof. The formulations produced contain an equivalent of 20-200 mg, preferably 20, 40, 80, 120, 160 or 200 mg of the free acid of the active substance. If the active substance is combined with HCTZ or chlorthalidone, the formulation contains 10-50 mg, preferably 50, 25 or 12.5 mg of the diuretic.

The advantageous activity of individual angiotensin II antagonists disclosed within the scope of this invention is particularly marked for the active substance telmisartan. If it appears useful or necessary to use an angiotensin II receptor blocker in conjunction with one or more other therapeutic active substances, telmisartan is a preferred angiotensin II receptor blocker, as it combines a blood pressure lowering and antidiabetic activity or helps to prevent diabetes. For this reason, preformulated active substance combinations of telmisartan with HMG-Co A reductase inhibitors such as simvastatin or atorvastatin constitute a major further development in the treatment of cardiovascular, cardiopulmonary, pulmonary or renal diseases, but also in the treatment of hyperlipidaemia, osteoporosis or Alzheimer's. This also applies to an active substance combination of telmisartan with rosiglitazone or pioglitazone or repaglinide or metformin or a DPP4 inhibitor in the treatment of diabetes. Telmisartan must also be regarded as a preferred RAS inhibitor in the treatment of high blood pressure with inhibitors of the renin-angiotensin system (RAS) combined with a calcium antagonist such as amlodipine or nifedipine or an aldosterone antagonist such as spironolactone or eplerenone. The combination with an aldosterone antagonist such as eplerenone also represents an important development in the treatment or prevention of weak heart or heart attack.

Therefore the present invention further relates to pharmaceutical compositions containing telmisartan or one of the salts thereof combined with

- amlodipine or nifedipine,
- eplerenone,
- simvastatin or atorvastatin,
- rosiglitazone or pioglitazone or repaglinide or metformin,
- a sulphonylurea,
- an aldosterone antagonist,
- an HMG-Co A reductase inhibitor or
- a DPP4 inhibitor,

and the preparation thereof. These combinations of active substances are generally incorporated with one or more formulation adjuvants such as mannitol, sorbitol, xylitol, saccharose, calcium carbonate, calcium phosphate, lactose, croscarmellose sodium salt (cellulose carboxymethylether sodium salt, cross-linked), crospovidone, sodium starch glycolate, hydroxypropylcellulose (low-substituted), maize starch, polyvinylpyrrolidone, copolymers of vinylpyrrolidone with other vinyl derivatives (copovidone), hydroxypropylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose or starch, magnesium stearate, sodium stearylfumarate, talc, hydroxypropylmethylcellulose, carboxymethylcellulose, cellulose acetate phthalate, polyvinyl acetate, water, water/ethanol, water/glycerol, water/sorbitol, water/polyethyleneglycol, propyleneglycol, cetylstearyl alcohol, carboxymethylcellulose or fatty substances such as hard fat or suitable mixtures thereof, into conventional galenic preparations such as plain or coated tablets, capsules, powders, suspensions or suppositories.

Tablets may be obtained for example by mixing the active substance or substances with one or more excipients and subsequently compressing them. The tablets may also consist of several layers. Examples of excipients are

- inert diluents such as mannitol, sorbitol, xylitol, saccharose, calcium carbonate, calcium phosphate and lactose;
- disintegrants such as croscarmellose sodium salt (cellulose carboxymethylether sodium salt, cross-linked), crospovidone, sodium starch glycolate, hydroxypropylcellulose (low-substituted) and maize starch;

- binders such as polyvinylpyrrolidone, copolymers of vinylpyrrolidone with other vinyl derivatives (copovidone), hydroxypropylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose or starch;
- lubricants such as magnesium stearate, sodium stearyl fumarate and talc;
- agents for achieving delayed release such as hydroxypropylmethylcellulose, carboxymethylcellulose, cellulose acetate phthalate and polyvinyl acetate; and
- pharmaceutically permitted colourings such as coloured iron oxides.

Examples:

Example 1: **Telmisartan, losartan and irbesartan do not bind *in vitro* to the PPARgamma ligand binding domain**

Protein containing the human PPARgamma-ligand binding domain (LBD) is prepared as a GST fusion protein in E.coli and purified by affinity chromatography.
To do this, a DNA section which codes for the amino acids 205-505 of the human PPARgamma2 transcription factor (cf. Genbank entry U79012) is subcloned via the additionally introduced restriction cutting sites BamH I and Xho I into the expression vector pGEX-4T-1 (Amersham) and the sequence of the section is monitored. The fusion protein is expressed in the *E.coli* strain BL21(DE3) recommended for pGEX vectors after induction with 0.2mM IPTG for 4 hours at 25°C. The bacteria are pelleted after the induction and frozen in batches in PBS, pH 7.4. After opening up in a French Press, the dissolved GST-PPARgamma-LBD-fusion protein is purified using a GSTrap column (Pharmacia). Elution is carried out by the addition of 20mM reduced glutathione.
The GST-PPARgamma-LBD-protein fractions are desalinated using a HiTrap desalting column (Pharmacia) and the protein concentration is determined using a standard assay.

Protein containing the human RXRalpha ligand binding domain (LBD) is prepared as a His tag fusion protein in *E.coli* and purified by affinity chromatography.
To do this a DNA section which codes for the amino acids 220-461 of the human RXRalpha transcription factor (cf. Genbank entry NM_002957, nt 729-1457) is subcloned via the additionally introduced restriction cutting sites BamH I and Not I into the expression vector pET28c (Novagen) and the sequence of the section is monitored. The fusion protein is expressed in the *E.coli* strain BL21(DE3) recommended for pET vectors after induction with 0.2mM IPTG for 4 hours at 25°C. The bacteria are pelleted after the expression and frozen in batches in PBS, pH 7.4. After opening up in a French Press, the dissolved His- RXRalpha -LBD-fusion protein is purified using a HiTrap chelating column (Pharmacia). Elution is carried out using a

500 mM imidazole step. The His-RXRalpha -LBD protein fractions are desalinated using a HiTrap desalting column (Pharmacia) and the protein concentration is determined using a standard assay.

a) AlphaScreen

Alpha Screen assays were first described in Ullmann EF et al, Proc Natl Acad Sci USA (1994) 91:5426-5430. The measurements carried out within the scope of this Example were carried out as described by Glickman JF et al., J Biomol Screen (2002) 7:3-10. The assay buffer consists of 25mM Hepes pH7.4, 100mM NaCl, 1mM DTT, 0.1% Tween-20, 0.1% BSA. 3nM GST-PPARgamma-LBD fusion protein, 15nM biotinylated LXXLL peptide of the cofactor CBP (corresponding to the peptide disclosed on page 218 of Mukherjee R et al., J Steroid Biochem Mol Biol (2002) 81:217-225 with an additional N-terminal cysteine), and in each case 10µg/ml of anti-GST-acceptor beads or streptavidine donor beads (Applied Biosystems) are incubated in a total volume of 12.5µl in the presence of different concentrations of a test substance (in DMSO) for 4 hours at ambient temperature. The final DMSO concentration in the assay is 1% (v/v). A 1% DMSO solution is used as the background control (NSB). The measurement is done using a Packard fusion measuring device.

	telmisartan		rosiglitazone	
conc. / M	MW	SD	MW	SD
NSB	619	21	573	17
1.00E-08			820	18
3.00E-08	642	41	1720	48
1.00E-07	606	10	8704	59
3.00E-07	644	56	27176	1232
1.00E-06	677	14	43233	1083
3.00E-06	720	35	52691	3771
1.00E-05	847	82	56366	4303
5.00E-05	1111	135		

Unlike rosiglitazone, a PPARgamma-agonist known from the literature with binding in the LBD, the use of increasing concentrations of telmisartan, losartan and irbesartan (concentrations of up to 50µM) does not result in any direct activation of the PPARgamma-LBD and hence in any significant recruiting of the LXXLL peptide.

b) SPA Assay

A description of the SPA assay format can be found in Mukherjee R et al., J Steroid Biochem Mol Biol (2002) 81:217-225. The assay buffer consists of 20mM Tris pH 7.5, 25mM KCl, 10mM DTT, 0.2% Triton X-100). 30nM GST-PPARgamma-LBD fusion protein, 30nM His-RXRalpha-LBD, anti-GST-antibody (1:600, Amersham Pharmacia), 0.25mg protein A SPA PVT antibody-binding beads (Amersham Pharmacia), 30nM ^{3}H-labelled rosiglitazone are incubated with dilutions of the test substance for 5 hours at room temperature in a total volume of 100µl.
10µM of unlabelled rosiglitazone is added as background control (NSB) instead of the radioactive rosiglitazone, and the solvent used, e.g. DMSO, is added as the maximum value (Bmax) instead of a test substance.
After the incubation the test preparations are centrifuged for 5 minutes at 2000 rpm in a Hettich Universal 30Rf centrifuge and measured using a Packard TopCount NXT.

	telmisartan		irbesartan		losartan	
conc / M	MW	SD	MW	SD	MW	SD
NSB	217	9	217	9	217	9
Bmax	911	15	911	15	911	15
1.00E-07	837	49	913	54	915	43
3.00E-07	802	28	810	49	835	11
1.00E-06	818	27	815	51	901	10
3.00E-06	818	20	779	26	814	53
1.00E-05	703	30	723	37	787	46
3.00E-05	691	222	648	40	784	96
1.00E-04	545	18	510	81	611	17

In contrast to direct PPARgamma-agonists which bind to the PPARgamma-LBD, no concentration-dependent displacement of the radioactive rosiglitazone from the binding pocket takes place even in the presence of very large excesses of telmisartan, losartan or irbesartan.

c) NMR investigations

In contrast to a direct PPARgamma ligand, e.g. rosiglitazone, no interaction of the test substance with amino acids in the binding pocket takes place during the measurement of the ^{15}N TROSY spectrum of the PPARgamma-LBD in the presence of the test substance telmisartan. The amino acids of the binding pocket have the same position in the presence of the test substances as in the absence of a ligand.

Example 2: **Preparation of a stably transformed PPARgamma reporter cell line**

A DNA section which codes for amino acids 205-505 of the human PPARgamma2 transcription factor (corresponding to nucleotides 703-1605 of Genbank sequence U79012) is incorporated into the Multiple Cloning Site of the vector pFA-CMV (Stratagene) via additionally introduced restriction cutting sites BamH I and Hind III and the sequence is verified. The resulting plasmid pFA-CMV/hPPARgamma2-LBD

codes N-terminally of the PPARgamma-LBD in the same reading frame for a Gal4 DNA binding domain. In addition the plasmid codes for a neomycin resistance.

The cell line CHO-K1 (ATCC CCL-61) is cotransfected with the plasmids pFA-CMV/hPPARgamma2-LBD and pFR-Luc (Stratagene). pFR-Luc codes for the luciferase gene under the control of a five-times repeated yeast Gal4 binding site. The transfection is carried out with lipofectamine2000 in accordance with the manufacturer's instructions.
After transfection the cells are cultivated in medium (Ham's F12 with 10% foetal calf serum) in the presence of 0.5 mg/ml G-418. After six days' cultivation the cells are passaged and kept in culture for another 10 days. The resulting neomycin-resistant colonies are picked out under the microscope and transferred into 96 well dishes and cultured. Various transformed cell lines are obtained with the plasmids contained therein (e.g. clone no. 10, 11, 13 etc), which are kept in the culture medium.

The cell lines are examined for the inducibility of the luciferase gene using a PPARgamma agonist, e.g. rosiglitazone, and react with an increased luciferase signal to stimulation by the PPARgamma agonist.

Example 3: **Telmisartan, losartan and irbesartan activate PPARgamma at cellular level**

The CHO-K1 cell line derived from the transformed clone 11 of Example 2 is seeded in 96-well flat-bottomed dishes in a density of $3x10^4$ cells/200µl/well and cultivated overnight in Ham's F-12 medium with 10% foetal calf serum and 0.5mg/ml G-418. After 24 hours the medium is changed for one without any added G-418.
The test substances are brought to 100 times the desired concentration with a suitable solvent, e.g. DMSO, and diluted 1:100 with the medium placed in the cell culture plate. The solvent used, e.g. DMSO, is used as the background control in the same concentration.

24 hours after the addition of the substance the supernatants are discarded and the cells are washed twice with 150µl washing buffer (25mM Tricine, 16.3mM $MgSO_4$, pH7.8). After the washing steps 50µl of washing buffer with 150µl of luciferase assay buffer (25mM Tricine, 0.5mM EDTA, 0.54mM NaTPP, 16.3mM $MgSO_4$, 1.2mM ATP, 0.05mM luciferine, 56.8mM 2-mercaptoethanol, 0.1% Trition X-100, pH7.8) are added to each test preparation. Luminescence is measured after a five minute wait using a Packard TopCount NXT. The luciferase activity is obtained by integrating the relative luciferase units (RLU) of the first ten seconds after the start of measurement.

	telmisartan		**irbesartan**		**losartan**		**rosiglitazone**	
conc / M	MW	SD	MW	SD	MW	SD	MW	SD
NSB	466	188	466	188	466	188	741	141
1.00E-08							2761	178
3.00E-08							8256	708
1.00E-07							35265	2947
3.00E-07	760	255	491	70	874	475	86859	6139
1.00E-06	2859	455	657	65	589	70	106252	30018
3.00E-06	24498	2290	1028	342	672	88	143232	14064
1.00E-05	61397	7853	3292	556	709	163	150989	24245
3.00E-05	58790	2055	22133	4202	3271	585		
1.00E-04			29600	6936	11322	1668		

The angiotensin II receptor antagonist telmisartan brings about a particularly potent activation of the PPARgamma pathway in the PPARgamma reporter cell line. Activation by other angiotensin II receptor antagonists such as losartan and irbesartan takes place only at higher test concentrations and to a lesser extent.

Example 4: **Examples of formulations**

Tablet 1

Tablets having the following composition are obtained by direct compression of the telmisartan sodium salt with excipients and magnesium stearate:

Ingredients:	mg
telmisartan sodium salt	41.708
mannitol	149.542
microcrystalline cellulose	50.000
croscarmellose sodium salt	5.000
magnesium stearate	3.750
total	250.000

Tablet 2

Tablets having the following composition are obtained by direct compression of the telmisartan sodium salt with excipients and magnesium stearate:

Ingredients:	mg
telmisartan sodium salt	83.417
sorbitol	384.083
polyvidone K25	25.000
magnesium stearate	7.500
total	500.000

Tablet 3

Hydrochlorothiazide, telmisartan sodium salt, sorbitol and red iron oxide are mixed in a free fall blender, passed through a 0.8 mm screen and, after the addition of magnesium stearate, processed in a free fall blender to obtain a powdered mixture.

This combination of active substances and excipients is than compressed with a suitable tablet press (e.g. Korsch EK0 or Fette P1200) to form tablets. Tablets with the following composition are obtained, the quantity of telmisartan sodium salt

contained in each tablet corresponding to a quantity of 80 mg of the free acid of telmisartan.

Ingredient	**mg/tablet**	**%**
telmisartan sodium salt	83.417	13.903
hydrochlorothiazide	12.500	2.083
sorbitol	494.483	82.414
red iron oxide	0.600	0.100
magnesium stearate	9.000	1.500
total	**600.000**	**100.000**

The telmisartan sodium salts of the tablets of the three batches dissolves in 900 ml of 0.1 M phosphate buffer, pH 7.5, at a rate of 92 ± 1.5 %, 96 ± 1.8 % and 100 ± 1.0 %, respectively, after 30 minutes' stirring (75 rpm). The hydrochlorothiazide dissolves in 900 ml of 0.1 M HCl (100 rpm) after 30 minutes at a rate of 69 ± 6.3 %, 72 ± 2.1 % and 78 ± 1.8 %, respectively.

Patent Claims:

1. Use of an angiotensin II receptor antagonist or one of the salts thereof for preparing a pharmaceutical composition for treating people in whom type 2 diabetes mellitus has been diagnosed or who are suspected of prediabetes.

2. Use according to claim 1, characterised in that in the subjects to be treated the fasting blood sugar level exceeds 125 mg glucose per dl of plasma.

3. Use according to claim 1, characterised in that in the subjects to be treated the fasting blood sugar level is 110-125 mg glucose per dl of plasma.

4. Use according to claim 1, characterised in that in the subjects to be treated a blood sugar level of more than 200 mg of glucose per dl of plasma is measured 2 hours after taking 75 g of glucose on an empty stomach.

5. Use according to claim 1, characterised in that in the subjects to be treated a blood sugar level of 140-200 mg of glucose per dl of plasma is measured 2 hours after taking 75 g of glucose on an empty stomach.

6. Use according to claim 1, characterised in that in the subjects to be treated the blood level for triglycerides exceeds 150 mg/dl.

7. Use according to claim 6, characterised in that in the subjects to be treated the blood level for HDL is less than 40 mg per dl of plasma in women and less than 50 mg per dl of plasma in men.

8. Use according to claims 6 und 7, characterised in that in the subjects to be treated the fasting blood sugar level exceeds 110 mg glucose per dl of plasma.

9. Use according to claims 1-8, characterised in that in the subjects to be treated the systolic blood pressure exceeds a value of 140 mm Hg and the diastolic blood pressure exceeds a value of 90 mm Hg.

10. Use according to claims 1, 2 und 4, characterised in that in the subjects to be treated the systolic blood pressure exceeds a value of 130 mm Hg and the diastolic blood pressure exceeds a value of 80 mm Hg.

11. Use according to claims 1-10, characterised in that in the subjects to be treated the ratio of waist measurement to hip measurement in women exceeds a value of 0.8 in women and a value of 1 in men.

12. Use according to claim 1, characterised in that the angiotensin II receptor antagonist has the property of activating the expression of a stably transfected luciferase gene after the addition of a stably transformed PPARgamma reporter cell line to the culture medium, without binding *in vitro* to the PPARgamma ligand binding domain.

13. Use according to claim 12, characterised in that
the angiotensin II receptor antagonist does not exhibit any binding *in vitro* to the ligand binding domain of a human PPARgamma receptor while
the angiotensin II receptor antagonist leads to the induction of a luciferase activity when it is added to the culture medium of a stably transformed cell line which
expresses a fusion protein consisting of the ligand binding domain of the human PPARgamma transcription factor and the yeast GAL4 DNA binding domain and
contains a luciferase gene under the control of a five-times repeated yeast Gal4 binding site.

14. Use according to claim 1, characterised in that the angiotensin II receptor antagonist is the active substance telmisartan.

15. Use according to claim 1, characterised in that the formulation of the pharmaceutical composition contains 20-200 mg telmisartan.

16. Use according to claim 1, characterised in that the angiotensin II receptor antagonist is combined with a diuretic.

17. Use according to claim 16, characterised in that the formulation of the pharmaceutical composition contains 10-50 mg of HCTZ or chlorthalidone.

18. Method of treating people in whom type 2 diabetes mellitus has been diagnosed or who are suspected of prediabetes, characterised in that a pharmaceutical

composition which contains an angiotensin II receptor antagonist is administered.

19. Method according to claim 18, characterised in that the angiotensin II receptor antagonist is the active substance telmisartan.

20. Pharmaceutical composition containing telmisartan in conjunction with

a) amlodipine or nifedipine,
b) eplerenone or spironolactone,
c) simvastatin or atorvastatin,
d) rosiglitazone or pioglitazone or repaglinide or metformin,
e) an aldosterone antagonist,
f) an HMG-Co A reductase inhibitor,
g) a DPP4 inhibitor or
h) a sulphonylurea.

Abstract

The invention relates to the use of angiotensin II receptor antagonists for treating people in whom type 2 diabetes mellitus has been diagnosed or who are suspected of prediabetes. Treatment is particularly indicated when there is a need to treat high blood pressure at the same time.